Docket No: 22740-2

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/Denise M. Everett/

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PATENT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Applicant:	Sándor Sipka et al	Confirmation No.:	8175
Serial No.:	10/651,136	Group Art Unit:	1644
Filed:	August 28, 2003	Examiner:	Rooney, Nora Maureen
For:	**Processes For Inhibiting Development of Allergic Disease**		

DECLARATION UNDER 37 C.F.R. 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Dr. Sándor Sipka declares that:

1. He is a co-inventor of and familiar with the present application Serial No. 10/651,136 filed on August 28, 2003, is familiar with the Official Action dated October 2, 2008, and the references cited therein, specifically, Cochran et al, "Influence of Lipopolysaccharide Exposure on Airway Function and Allergic Responses in Developing Mice," *Pediatric Pulmonology*, 34:267-277 (2002), Previte et al, "Detoxification of *Salmonella typhimurium* Lipopolysaccharide by Ionizing Radiation," *Journal of Bacteriology*, 93(5):1607-1614 (1967), and Khan et al, "Functional and immune response to lipopolysaccharide and allergens in developing mice," *Pediatric Research*, 51:474A, (2002).

2. He holds the position of Chief of the Regional Immunological Laboratory, Third Department of Internal Medicine, University of Debrecen, Research Center for

Molecular Medicine, Medical and Health Science Center, Debrecen, Hungary and is knowledgeable in the art of immunology.

3. The present invention is based on the discovery of a unique immune response elicited by irradiation-detoxified (IR) lipopolysaccharide (LPS) and the use of the IR-LPS in a method of decreasing development of allergic asthma by exposing a neonatal or immature mammal to the IR-LPS. To demonstrate the unexpected and surprising results of the present methods, the experiments described herein were conducted under his direction and control to test whether IR-LPS exhibits characteristics different from native LPS with respect to in vivo immunomodulatory allergy-prevention.

4. As part of a pre-treatment regimen, water and equal concentrations of native LPS and IR-LPS were sprayed for 8 weeks into the cages of infant mice (age at onset = 6 weeks, Balb/c mice). At the end of the pretreatment period the animals were sensitized by two intraperitoneal injections of 150 μg ragweed allergen (RWE). On day 11 following the sensitization treatment they were challenged with 100 μg RWE. Three days later the cell counts (macrophages and neutrophils/ml) of bronchial lavage (BAL) were measured as well as the serum concentrations of TNFα, a Th1 type cytokine (determined by ELISA). The concentrations of cytokines IL-4 and IL-5 (a Th2 cytokine) were also measured but concentrations for all but IL-5 in the IR-LPS group were below detection limits.

5. The measured results were as follows:

Table 1. The average number of inflammatory neutrophil granulocytes in the bronchial lavage fluid of ragweed sensitized mice after allergen challenge following daily pre-treated with water (H_2O), LPS or IR-LPS sprays for 8 weeks.

Groups of animals treated with	Total number of cells (macrophages + neutrophils)(cells/ml lavage)	Number of neutrophils (cells/ml lavage)
H_2O spray (controls) (5 ml/day) (n=5)	84750	26273

IR-LPS spray (5 µg/5ml H_2O /day) (n=5)	72000* p= 0.04	15840* p = 0.02
LPS-spray (5 µg/5ml H_2O /day) (n=5)	76500	24480

Table 2. The average concentration of tumor necrosis factor alpha (TNFα) (Th1 type cytokine) and interleukin 5 (IL-5) (Th2 type cytokine) in sera of infant Balb/c female mice after an allergic reaction by ragweed following daily pre-treated with water (H_2O), LPS or IR-LPS sprays for 8 weeks

Groups of animals treated with	TNFα (Th1 type cytokine) pg/ml	IL-5 (Th2 type cytokine) pg/ml
H_2O spray (controls) (5 ml/day) (n=5)	4.60	undetectable
IR-LPS spray (5 µg/5ml H_2O /day) (n=5)	16.31* p = 0.001	2.19
LPS-spray (5 µg/5ml H_2O /day) (n=5)	7.60	undetectable

6. The results set forth in the above Table 1 demonstrate that during the allergic reaction to ragweed, the number of inflammatory neutrophils was significantly decreased in the group of mice treated with IR-LPS ($p = 0.02$) compared to those treated with either H_2O spray or native LPS. The results set forth in Table 2 demonstrate that during the ragweed-specific allergic reaction the serum level of TNFα (Th1 type cytokine) was increased significantly by 3.56 fold ($p = 0.001$) compared to the controls (16.31/4.58=3.56). However the effect of native LPS was only 1.66 fold (7.60/4.58=1.66). These results illustrate a striking difference between the in vivo immunomodulatory effects of IR-LPS and native LPS on macrophage and neutrophil numbers. This indicates that the prolonged pre-treatment of the environment of infant mice with IR-LPS acts to prevent the intensity of ragweed specific allergic reaction differentially when compared to native LPS. Furthermore, it is clear that IR-

LPS caused a significant increase in the serum concentration of TNFα compared to LPS. It is his opinion that the surprisingly marked difference between the in vivo effects of IR-LPS and native LPS is due to their differing antigenic character which acts on the immunomodulatory Th1 type cells having antigen-specific T cell receptors. It is further his opinion that irradiation of LPS with 150 kGY 60Co-gamma ray results in production or revelation of a new or formerly hidden antigenic determinant(s) in the components of IR-LPS lending the altered and different antigenic character as clearly demonstrated in this study.

7. The surprisingly superior effect of IR-LPS over native LPS in protecting against the development of a hyper-immune response to an allergen is neither taught nor suggested by any of the prior art cited in the Official Action as noted above or otherwise known to me. Thus, none of this prior art, alone or in combination, suggests any benefit of using IR-LPS, particularly as compared with native LPS, in a method of decreasing development of allergic asthma. Accordingly, none of this prior art, alone or in combination, suggests a method of decreasing development of allergic asthma by exposing a neonatal or immature mammal to IR-LPS.

8. He further declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully Submitted,

23/February/2009
Date

Sándor Sipka

Sándor Sipka